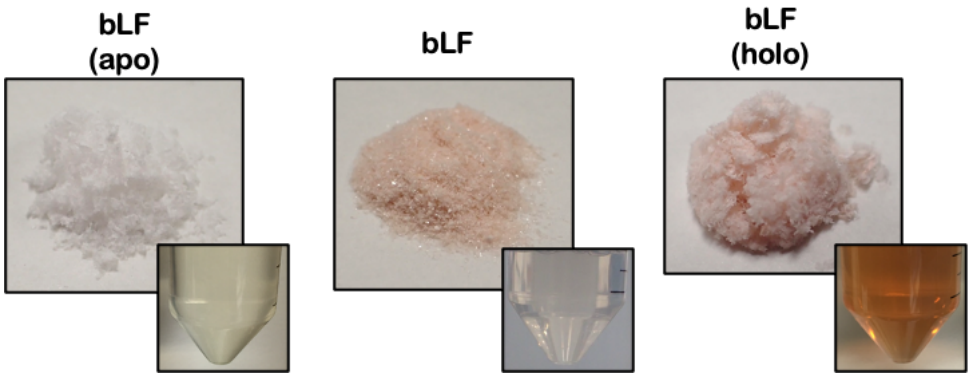
Bovine lactoferrin inhibits *Plasmodium berghei* growth by binding to heme

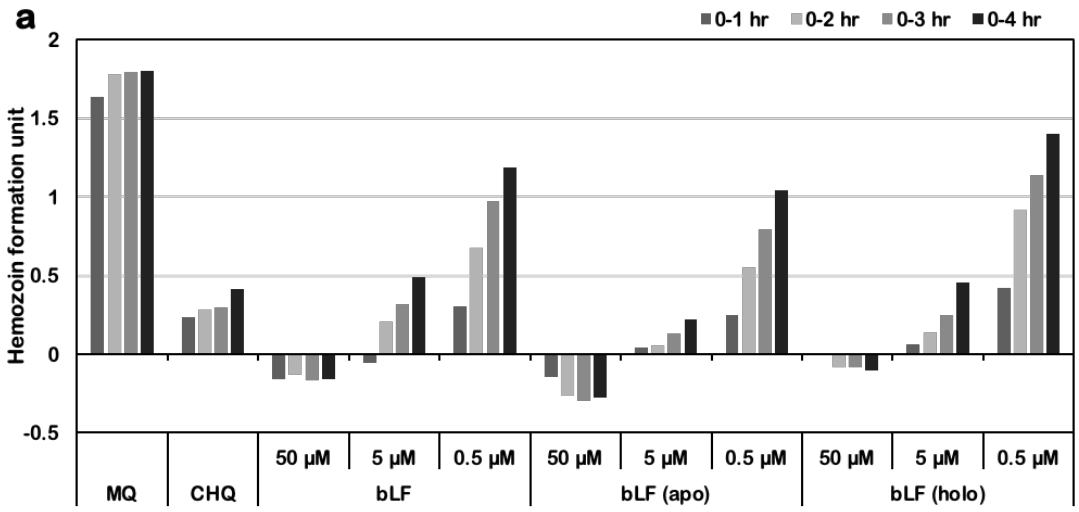
Supplementary Figures

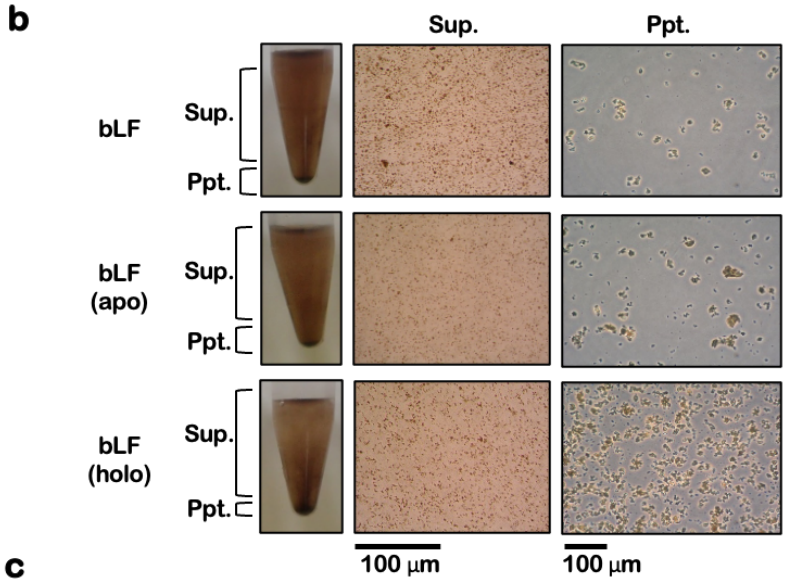


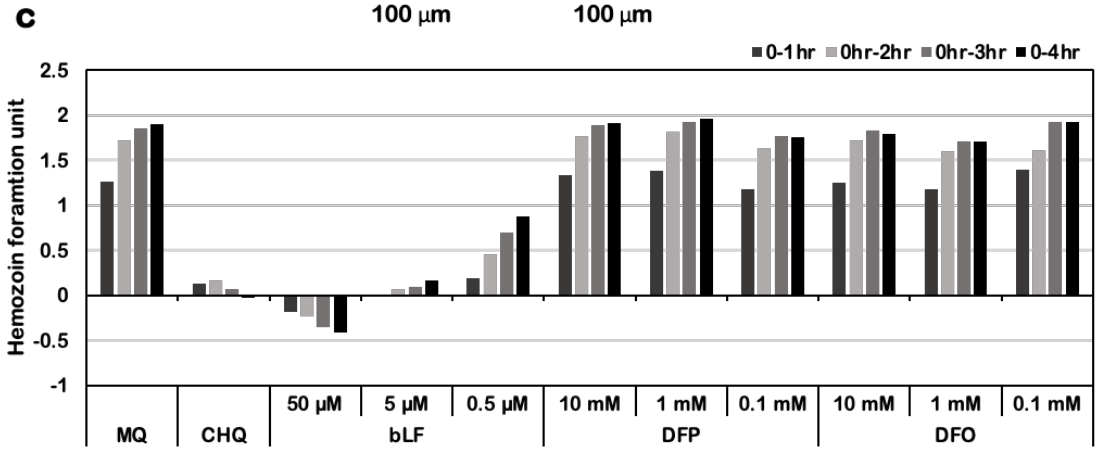
Supplementary Figure S1. Preparation of apo and holo bLF powders was conducted, with the subsequent observation of their coloration upon dissolution.

Supplementary Table S1. Iron content of bLF in different states.
Two iron atoms can be bound to one molecule of bovine lactoferrin, estimated to be fully saturated when the binding molar ratio is 200%.

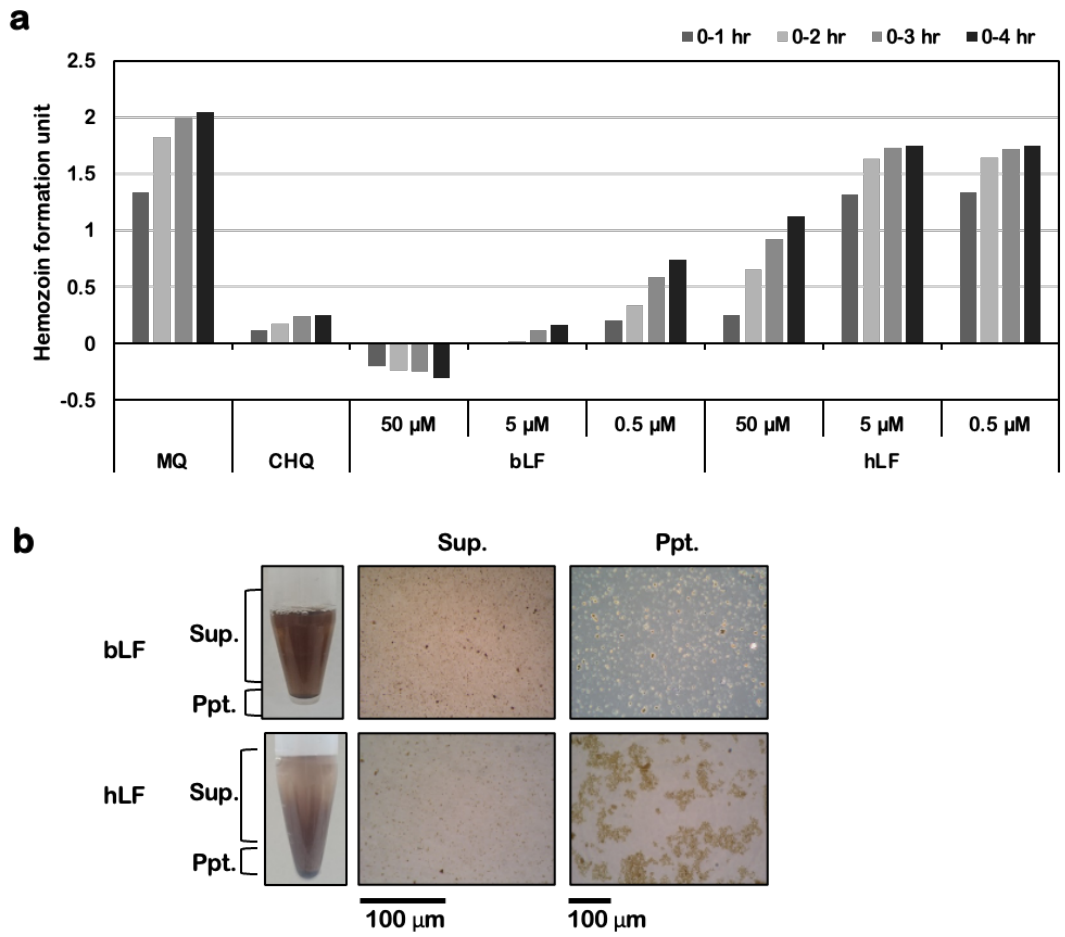
	bLF (Native)	Apo-	Holo-
Fe-bonded molar ratio (%)	16.1	6.75	145.0


a
0-1 hr
0-2 hr
0-3 hr
0-4 hr
Hemozoin formation unit
2
1.5
1
0.5
0
-0.5
MQ
CHQ
50 μM
5 μM
0.5 μM
bLF
50 μM
5 μM
0.5 μM
bLF (apo)
50 μM
5 μM
0.5 μM
bLF (holo)

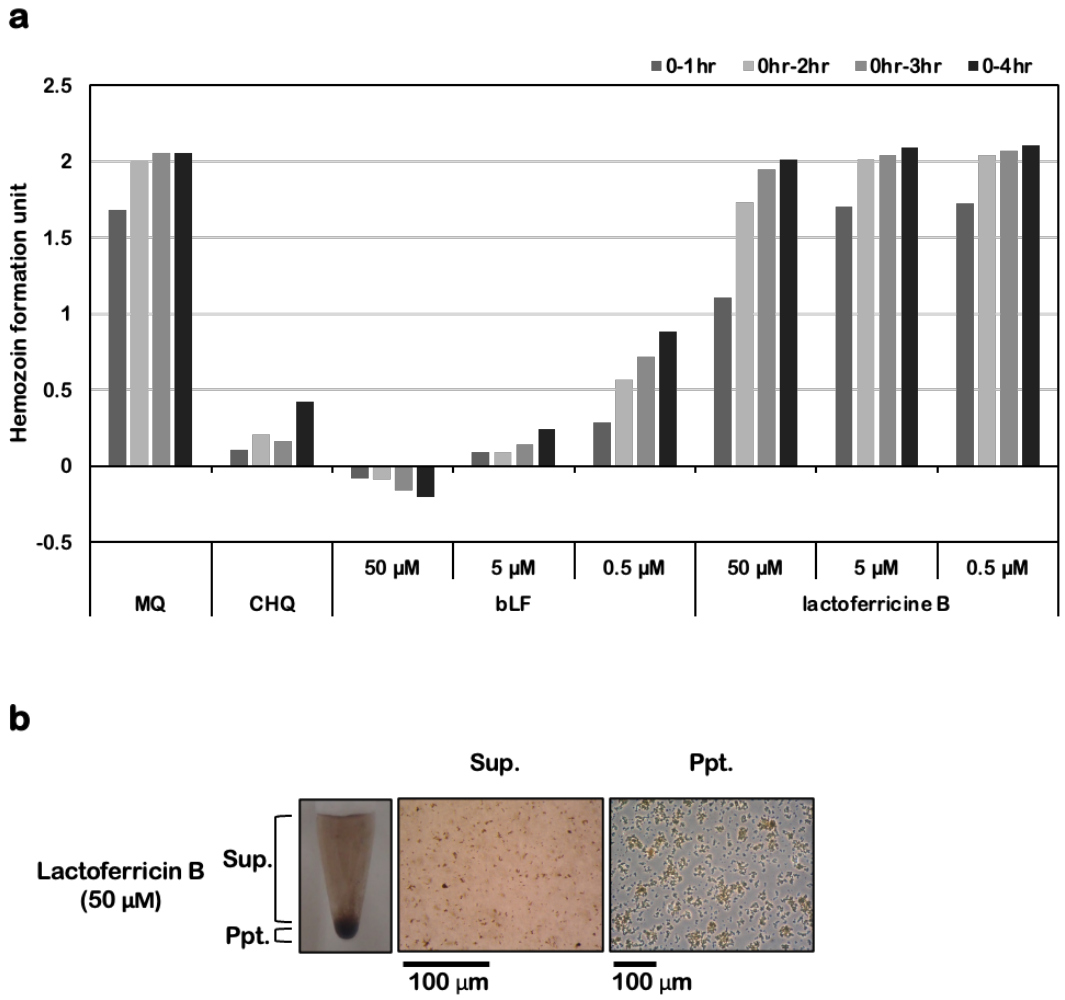

b
Sup.
Ppt.
bLF
Sup.
Ppt.
bLF
(apo)
Sup.
Ppt.
bLF
(holo)
Sup.
Ppt.
100 μm
100 μm


c
0-1hr
0hr-2hr
0hr-3hr
0-4hr
Hemozoin foramtion unit
2.5
2
1.5
1
0.5
0
-0.5
-1
MQ
CHQ
50 μM
5 μM
0.5 μM
bLF
10 mM
1 mM
0.1 mM
DFP
10 mM
1 mM
0.1 mM
DFO

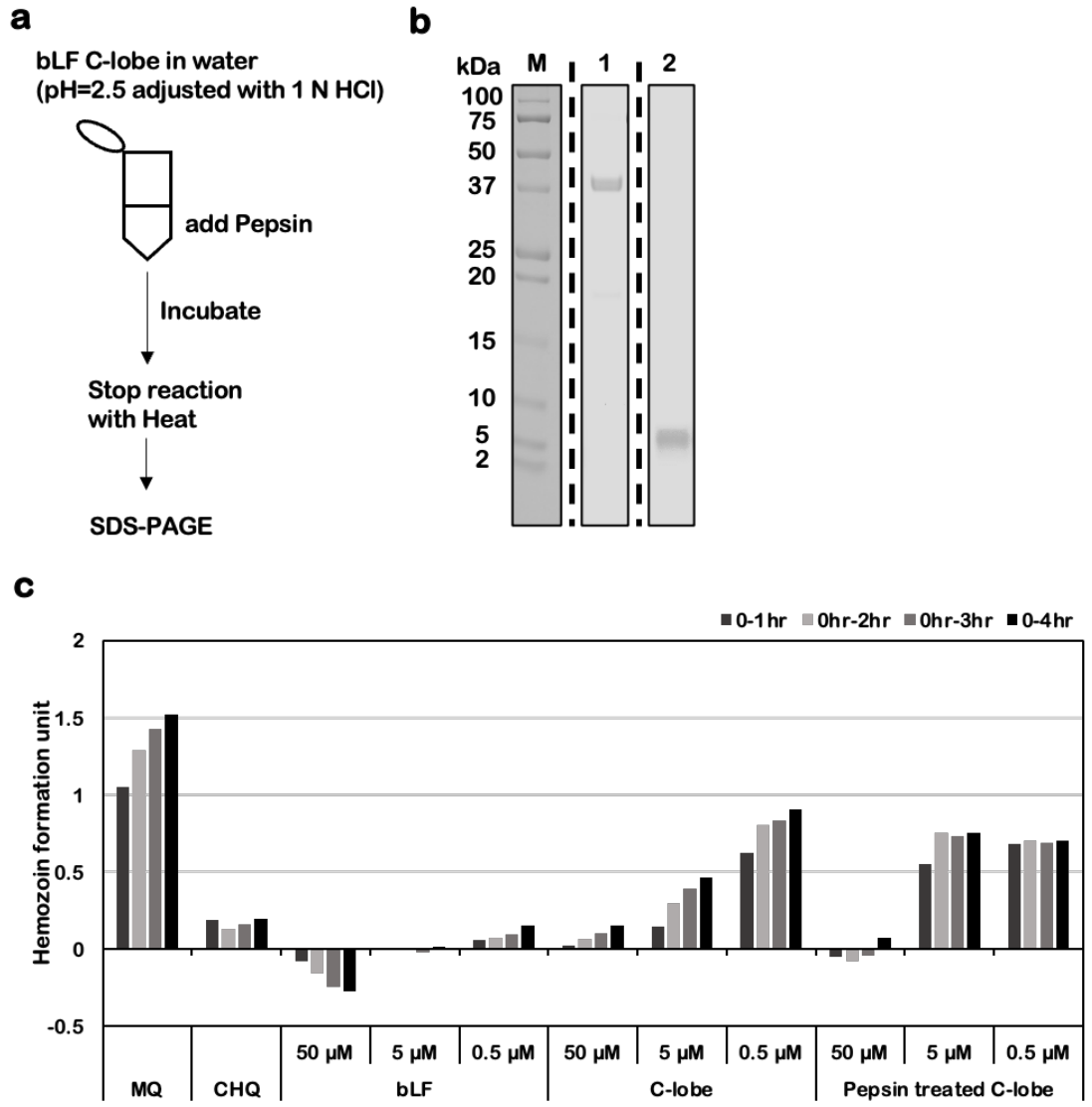
Supplementary Figure S2. Evaluation of the functional roles of lactoferrin in its various iron-bound states: (a) inhibition of hemozoin formation, and (b) degradation of hemozoin. (c) The ability of iron chelators to inhibit hemozoin formation. For (a) and (c), various bLF forms were tested at 0.5, 5, and 50 μM concentrations, while DFP and DFO were tested at 0.1, 1, and 10 mM. All tests were conducted in triplicate. In (b), samples were standardized to a 50 μM concentration. The microscope field of view presents a representative image.



Supplementary Figure S3. Assessment of the ability to inhibit hemozoin formation (a) and hemozoin degradation (b) of human lactoferrin (hLF). For (a), hLF were tested at 0.5, 5, and 50 μM concentrations. All tests were conducted in triplicate. In (b), hLF was standardized to a 50 μM concentration. The microscope field of view presents a representative image.



Supplementary Figure S4. Assessment of the ability to inhibit hemozoin formation (a) and hemozoin degradation (b) of lactoferricin B. For (a), lactoferricin B were tested at 0.5, 5, and 50 μM concentrations. All tests were conducted in triplicate. In (b), lactoferricin B were standardized to a 50 μM concentration. The microscope field of view presents a representative image.

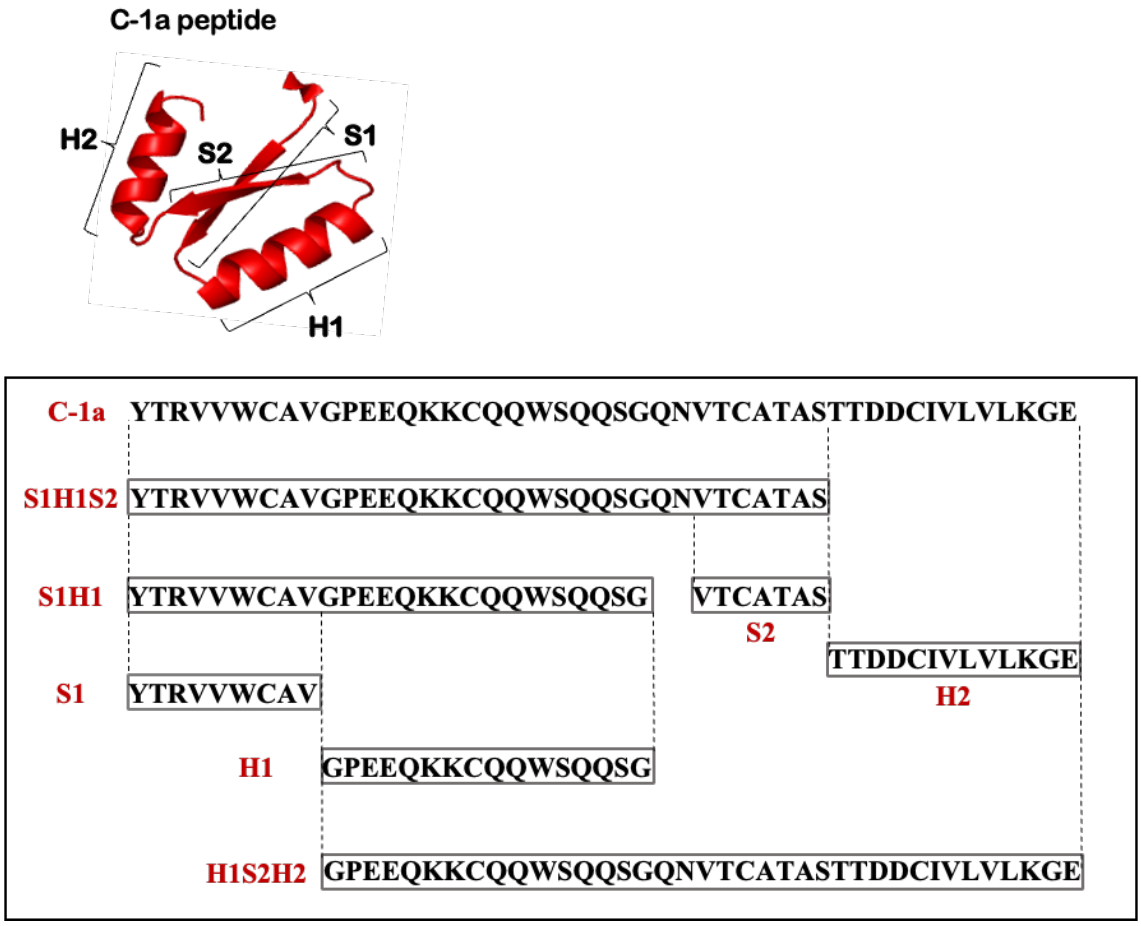


Supplementary Figure S5. Evaluation of degradative product of C-lobe.

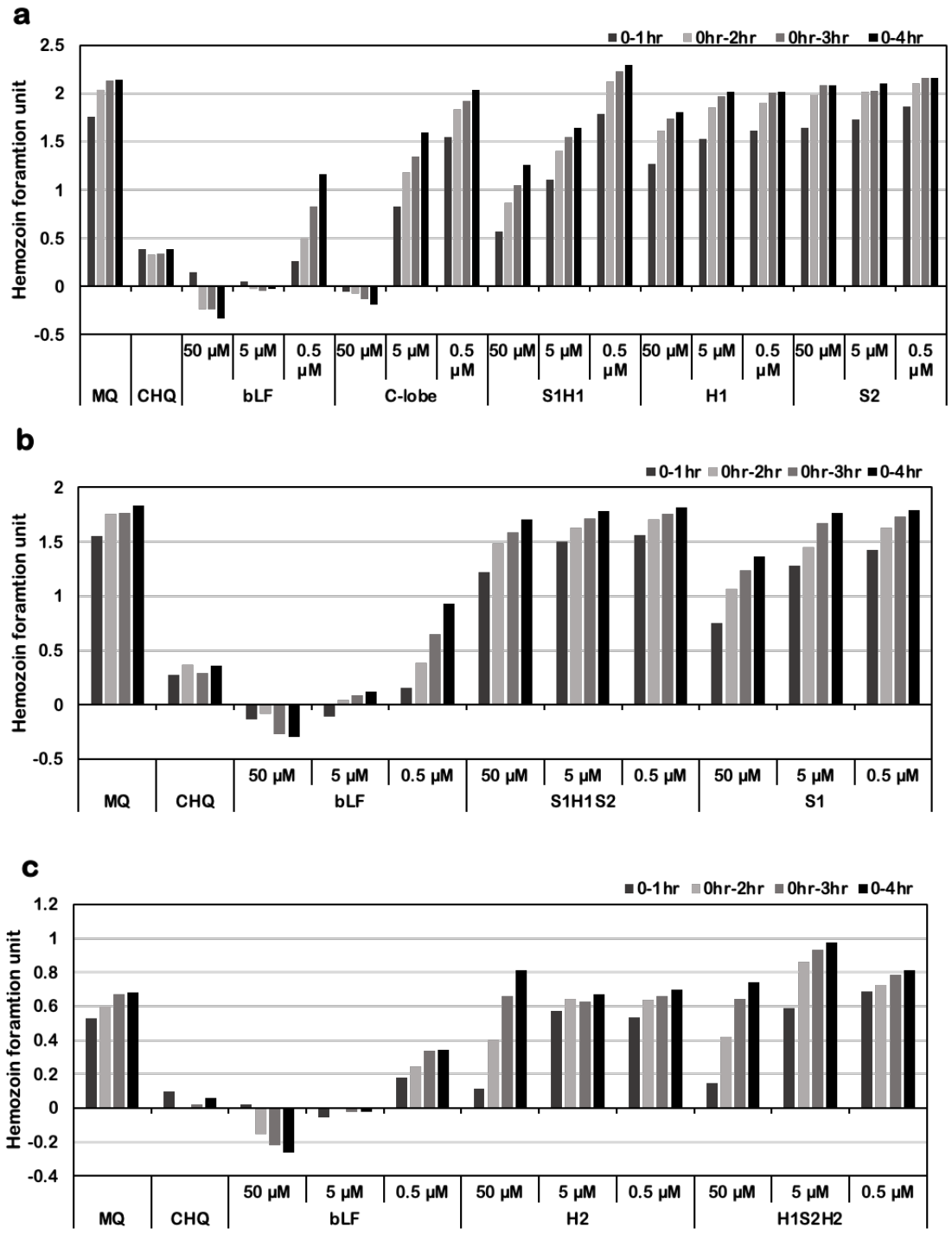
(a) Overview of pepsin degradation reaction of C-lobe. (b) 12.5% Tris-Tricine-SDS-PAGE/CBB of pepsin degradation products of C-lobe. M, molecular weight, lane 1: intact C-lobe, lane 2: pepsin-treated C-lobe. (c) Assessment of the ability to inhibit hemozoin formation of pepsin-treated C-lobe. Various samples were tested at 0.5, 5, and 50 μM concentrations. All tests were conducted in triplicate.

Supplementary Table S2. Amino acid sequence of each region and molecular weight.

Region	Sequence	Molecular mass
C-1a	YTRVVWCAVGPEEQKKCQQWSQQSGQNVTCATASTTDDCIVLVLKGE	5189.74
C-2	SQSCAPGADPKSRLCALCAGDDQGLDKCVPNSKEKY	3756.18
C-1b	DRAAHVEQVLLHQQALFGKNGKNCPDKFCLF	3528.02
Hinge	DSALYLGSRYLTTLKNLRETAEEVKARYTRVVW	3903.39



Supplementary Figure S6. Secondary structures and amino acid sequences comprising the C-1a region. The structural model of the C-1a region is described at the top. Each secondary structure is defined as S1, H1, S2 and H2. The sequence and positional relationship of each are described at the bottom.



Supplementary Figure S7. (a-c) Assessment of the ability to inhibit hemozoin formation of various region derived from C-1a. Samples were teste at 0.5, 5, and 50 μM concentrations. All tests were conducted in triplicate.

Supplementary Table S3. Inhibition ratio of hemozoin formation.

The values show the relative effects of the various substances in the hemozoin formation inhibition experiment, with the effect of CHQ at hour 4 as 100%.

Sample	Hemozoin formation inhibition effect (%)
CHQ	100
bLF	138.8
C-lobe	103.6
C-1a	103.5
S1H1S2	8.4
S1H1	44.2
H1S2H2	0
S1	31.6
H1	19.0
S2	0
H2	0